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Simple Syntheses of Malathion and Malaoxon Enantiomers, and Isomalathion Diastereoisomers: Toxicity–Configuration Relationship

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Abstract: Malathion enantiomers were synthesized by nucleophilic substitution of *O*,*O*-dimethyl dithiophosphoryl anion to diethyl (*R*)- or (*S*)-2-bromosuccinate. Malaoxon enantiomers were obtained from optically active malathions in thiono–thiolo rearrangement with 65% HNO_3. Desmethylation of malathion enantiomers by triethylamine, following the remethylation using methyl iodide gave isomalathion diastereomeric pairs. Physicochemical characteristics of the compounds obtained, and their influence on rats and some species of arthropods, are presented. © 1998 SCI

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Key words: malathion, malaoxon, isomalathion, enantiomers, synthesis, toxicity

1 INTRODUCTION

Malathion [*S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate (Fig. 1, **1**) is one of the most widely used insecticides. Besides its high efficacy against arthropods, it has the advantage of low toxicity to mammals. This feature is due to the activity of carboxylesterases—detoxifying enzymes, which hydrolyse the ester groups in the succinic ligand of malathion to α- and β-monoacids, which are easily removed in urine as metabolites.[1–4] In insects, which lack this kind of enzyme, malathion toxicity is much higher.[4]

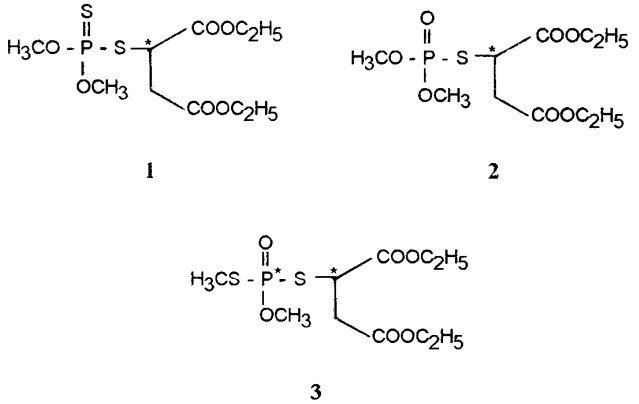

Fig. 1. Structures of Malathion (**1**), Malaoxon (**2**) and Isomalathion (**3**). ★ = Chiral centres.

Malaoxon [*S*-1,2-di(ethoxycarbonyl)ethyl *O*,*O*-dimethyl thiophosphate] (**2**) is an impurity present in technical grade malathion.[5] It is also one of the possible metabolites created during the enzymatic oxidation of malathion.[6] Malaoxon toxicity to mammals is higher than malathion toxicity (e.g. LD_{50} p.o. of malaoxon for rats, 158 mg kg^{-1}; LD_{50} p.o. for rats of malathion, 12 500 mg kg^{-1}).[5,7] This feature is due to the ability of malaoxon to inhibit the acetylcholinesterase enzyme responsible for acetylcholine hydrolysis.[6] In addition, malaoxon also inhibits the malathion carboxylesterase.[5]

Isomalathion [*S*-1,2-di(ethoxycarbonyl)ethyl *O*,*S*-dimethyl dithiophosphate] (**3**) is, like malaoxon, one of technical-grade malathion impurities, created by thermal or photochemical isomerization.[8] In contrast to the low toxicity of malathion, isomalathion is highly toxic to mammals (about 1000-fold more potent anticholinesterase than malathion).[8] Isomalathion and some other impurities of technical malathion caused the

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dangerous intoxication of workers involved in a mosquito control operation in Pakistan in 1976.[9]

The effect of stereoselectivity of biologically active compounds on the enzymes catalysing the basic reactions in organisms is well known. Malathion and malaoxon molecules have a chiral centre—an α-carbon atom in the succinic part of their structure. The isomalathion molecule has two chiral centers: the α-carbon atom, and the phosphorus atom.

In spite of the widespread use of malathion and the large amount of information concerning its biological mode of action, the first total synthesis of malathion enantiomers was published by Berkman and Thompson only in 1992.[10] It was followed by the detailed experimental procedure in 1993.[11] Earlier, in 1968, Hassan and Dauterman had described the synthesis of *O*,*O*-diethyl malathion analogue enantiomers.[12]

Both the above-mentioned methods required optically active substrates: diethyl (*R*)- and (*S*)-2-bromosuccinate[12] or diethyl (*R*)- and (*S*)-2-(trifluoromethylsulfonyloxy)succinate.[10,11] These substrates underwent nucleophilic substitution reactions with *O*,*O*-dimethyl or *O*,*O*-diethyldithiophosphoryl anions. As a result, enantiomers of *O*,*O*-diethyl malathion analogues were obtained with 57%,[12] and malathion enantiomers with 80% yield.[11]

Conversion of malathion into malaoxon by use of nitric acid as the oxidizing agent started to be the subject of research works in 1950s.[13,14] The route consisted of the direct treatment of malathion with excess of 70% nitric acid, keeping the reaction temperature between 25 and 31°C. In this method malaoxon was obtained in 62% yield.

The first synthesis of malaoxon enantiomers was made by Jackson and Berkman[15] from the respective stereoisomers of malathion by oxidative desulfuration using magnesium monoperphthalate (MMPP). As a result of this stereoselective and chemoselective oxidation, malaoxons were obtained in 50–55% yield.

Hassan and Dauterman[12] have also described the method of preparation of *O*,*O*-diethyl malaoxon analogue enantiomers (yield 42–44%) by the reaction of diethyl (*R*)- or (*S*)-2-bromosuccinate with the sodium salt of *O*,*O*-diethylthiophosphoric acid.

Berkman *et al.*[11] have described the method of obtaining isomalathion stereoisomers, in the first step going through the dealkylation of malathion enantiomers by strychnine, and then separation of the mixture of diastereomeric pairs by fractional repeated crystallization. Realkylation of separated methylstrychninium salts of desmethyl (*R*)- and (*S*)-malathions led to four isomalathion stereoisomers with maintained configuration at the carbon atom, and configuration at phosphorus appropriate to its configuration in the separated salts.

The aim of the authors of this paper was to obtain malathion and malaoxon enantiomers, as well as isomalathion diastereomers pairs, in an economically effective way, to obtain these compounds in quantities sufficient for research into their toxicological properties (evaluation of differences in their effects towards mammals, and some species of arthropod).

2 MATERIALS AND METHODS

2.1 Materials

L-Aspartic acid, D-aspartic acid (ex. Fluka)
Maleic anhydride (ex. Chemical Factory—Blachownia Śląska)
Phosphorus pentasulfide (ex. Montedison)

All solvents used were bought at POCh (Poland), and each of them was distilled before using in the syntheses.

2.2 Analytical methods

Melting points were measured on electrothermal apparatus. TLC was carried out on aluminium plates (20 × 20 cm) covered with silica gel Kieselgel 60 F_{254} (Merck), eluant: hexane + ethyl acetate (8 + 2, 6 + 4 by volume); detection: UV, 1% $PdCl_2$ in methanol. Column chromatography was carried out on silica gel 70–230 mesh (Merck).

GC analysis: Varian type 3300 apparatus equipped with flame—ionization detector, and split-splitless type injector (column: DB-1·15 m × 0·53 mm, film thickness 1 μm). GC/MS analysis: Varian type 3300 apparatus coupled to ITD 800 Finnigan MAT detector. H(200 MHz), ^{13}C(50 MHz), and ^{31}P(81 MHz). NMR analysis: Varian type Gemini 200 apparatus, internal standard tetramethylsilane, solvents deuterochloroform or hexadeuterodimethyl sulfoxide (for 2-bromosuccinic acids).

Optical rotatory measurement: JASCO digital polarimeter type DIP 380; Carl Zeiss Jena polarimeter.

Chiral HPLC analysis: Varian type 5000 apparatus with gradient pump, UV detector with gradient wavelength, and 4270 type integrator; Chiralcel OJ Daicel column (25 cm × 4·6 mm ID), liquid phase: hexane + 2-propanol (95 + 5 by volume for analyses of malaoxon and isomalathion, and 93 + 7 by volume for malathion analysis); $\lambda = 220$ nm; temp. 40°C; flow rate 1 ml min^{-1}; Chiral Si 100-L-Hypro Cu Serva column (25 cm × 4·6 mm ID), liquid phase: 0·001 M or 0·003 M aqueous copper sulfate (for 2-bromosuccinic acid analysis), $\lambda = 254$ nm, room temperature, flow rate 2 ml min^{-1}.

2.3 Syntheses

2.3.1 Malathion enantiomers

The synthesis of malathion enantiomers was undertaken using diethyl bromosuccinate as a substrate. This method, being a modification of the synthetic route

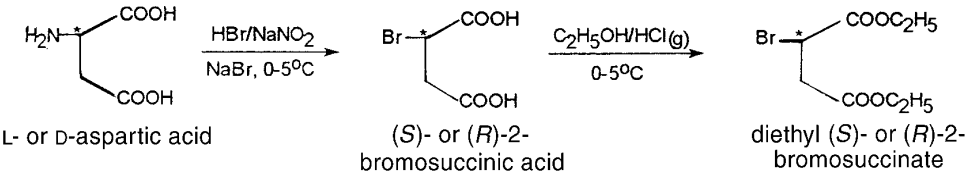


Fig. 2. Synthesis of diethyl 2-bromosuccinate enantiomers.

applied by Hassan and Dauterman,[12] requires three stereochemical operations on the chiral carbon, but is effective, and each of the steps can be realized in mild conditions.

For the synthesis of (*R*)-malathion, we used diethyl (*S*)-2-bromosuccinate, obtained by esterification of (*S*)-2-bromosuccinic acid with ethanol.[16,17] (*S*)-2-Bromosuccinic acid was synthesized from L-aspartic acid by diazotization in the presence of sodium bromide.[18] This conversion takes place with the maintenance of configuration.[19] D-Aspartic acid undergoes the same sequence of reactions as L-aspartic acid, resulting in diethyl (*R*)-2-bromosuccinate. The synthetic route is shown on Fig. 2.

Racemic bromodiester was synthesized from maleic anhydride by esterification with ethanol, following the addition of hydrogen bromide to the carbon–carbon double bond.[20,21] Physicochemical properties of these intermediates in the malathion enantiomer and racemate syntheses are given in Table 1.

The second substrate, essential for the preparation of malathion and its enantiomers, the ammonium salt of *O,O*-dimethyldithiophosphoric acid, was obtained using the Malatesta and Pizotti method, which utilizes P_2S_5 and methanol.[22] Neutralization of the synthesized acid with gaseous ammonia gave the required salt with a yield of 54% (m.p. 148–149°C) after crystallization from ethyl acetate.

The racemic diethyl 2-bromosuccinate was reacted with one of the alkaline salts of *O,O*-dimethyldithiophosphoric acid, sodium, potassium or ammonium, in different solvents. These experiments resulted in racemic malathion with a satisfactory yield of 50–60% after purification by column chromatography. The optimal conditions for reaction were determined as follows: reagents, diethyl 2-bromosuccinate and ammonium salt of *O,O*-dimethyldithiophosphoric acid; solvent, 1,4-dioxane; reaction time, 8 h; temperature, 50–55°C. Similar reaction conditions were applied during the syntheses of (*R*)- and (*S*)-malathion enantiomers and resulted in good yields and stereoselectivity.

The synthesis of malathion enantiomers is shown in Fig. 3, and their physicochemical properties are given in Table 2. Figure 4 represents the resolution of racemic malathion into its enantiomers, (*R*)-, and (*S*)-malathion.

TABLE 1
Characteristics of Intermediates

Compound	*Yield* (%)	*B.p. or m.p.** (°C)	*GC* (%)	*Enantiomeric excess*(%)	n_D^t	$[\alpha]_D^t$	R_f (*hexane + EtOAc; 8 + 2*)
(*R*)-2-Bromosuccinic acid	84·2	178–180* (dec.)	—	pure in the analysis conditions	—	$+70{\cdot}1^{18}$ (c = 6, EtOAc)	—
(*S*)-2-Bromosuccinic acid	90·0	177–180* (dec.)	—	pure in the analysis conditions	—	$-74{\cdot}1^{22}$ (c = 6, EtOAc)	—
Diethyl (*R*)-2-bromosuccinate	74·0	68/0·008 mm Hg	99·0	not analysed	$1{\cdot}4510^{18}$	$+45{\cdot}0^{18}$ (c = 6, EtOAc)	0·58
Diethyl (*S*)-2-bromosuccinate	64·7	68/0·008 mm Hg	99·0	not analysed	$1{\cdot}4522^{22}$	$-43{\cdot}3^{18}$ (c = 6, EtOAc)	0·58
Diethyl maleate	91·0	58/0·6 mm Hg	98·0	—	$1{\cdot}4418^{20}$	—	0·47
Diethyl (*RS*)-2-bromosuccinate	90·1	70/0·008 mm Hg	99·0	—	$1{\cdot}4592^{19}$	—	0·58

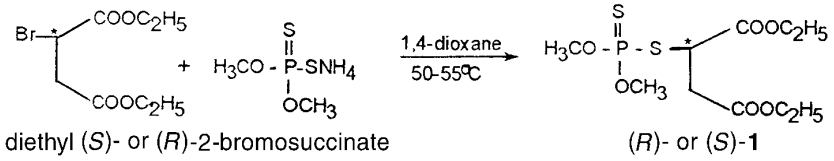


Fig. 3. Synthesis of malathion enantiomers.

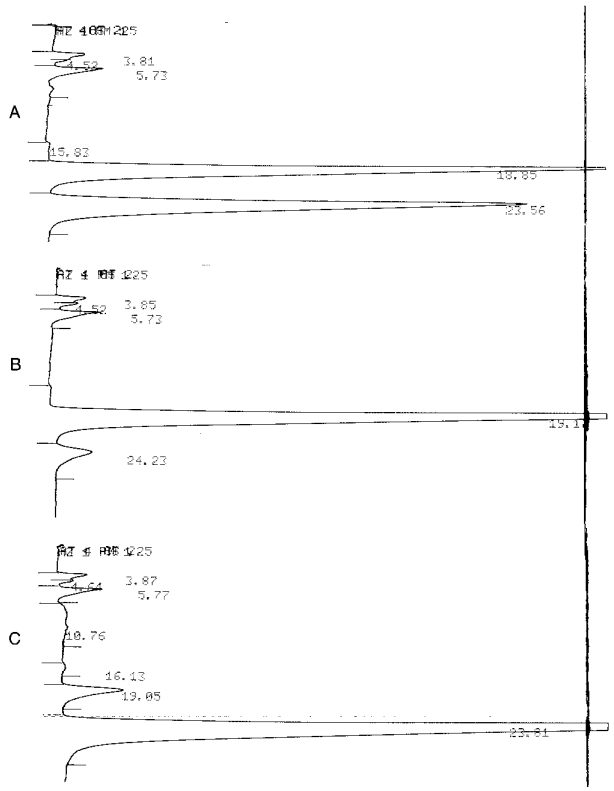


Fig. 4. Chiral HPLC resolution of (A) racemic malathion, and chromatograms of (B) (*R*)- and (C) (*S*)-malathion enantiomers.

2.3.2 *Malaoxon enantiomers*

Malaoxon enantiomers, as well as its racemate, were obtained by thiono–thiolo rearrangement of malathion using 65% technical grade nitric acid as an oxidizing agent (Fig. 5) in the amount of 7-fold molar excess, comparing to malathion. The reaction gave good yield and stereoselectivity (estimated by chiral HPLC, Fig. 6).

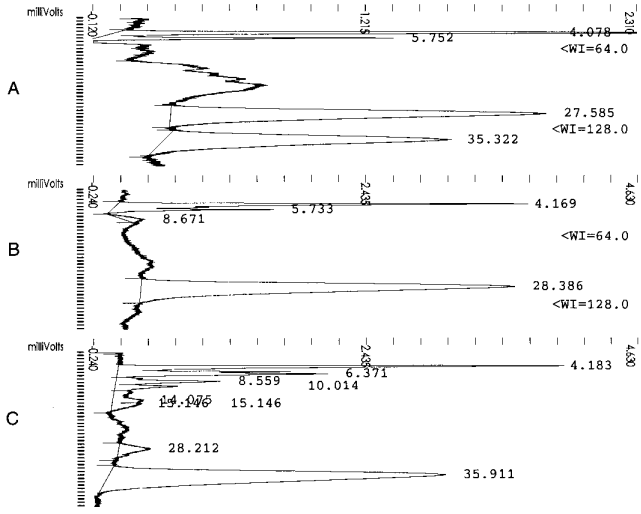
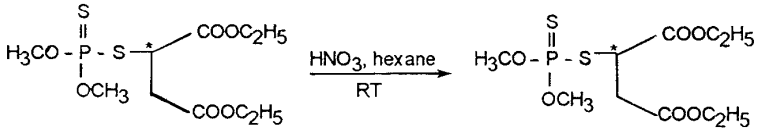


Fig. 6. Chiral HPLC resolution of (A) racemic malaoxon, and chromatograms of (B) (*R*)-, and (C) (*S*)-malaoxon enantiomers.



(*RS*)-, (*R*)- or (*S*)-**1** (*RS*)-, (*R*)- or (*S*)-**2**

Fig. 5. Synthesis of malaoxon enantiomers.

TABLE 2
Characteristics of Malathion and Malaoxon Enantiomers, and Isomalathion Diastereoisomers

Compound	*Yield* (%)	*GC* (%)	$[\alpha]_D$	n_D^t	*Enantiomeric excess (e.e)* (%)	R_f (*hexane EtOAc*)
(*RS*)-Malathion	60·0	99·0		$1{\cdot}4991^{20}$		0·34 (8 + 2)
(*R*)-Malathion	50·0	99·0	$+81{\cdot}2^{20}$ (c = 1·08, $CHCl_3$)	$1{\cdot}4995^{20}$	88·06	0·34 (8 + 2)
(*S*)-Malathion	56·0	98·0	-80.0^{20} (c = 1·03, $CHCl_3$)	$1{\cdot}4990^{20}$	90·08	0·34 (8 + 2)
(*RS*)-Malaoxon	54·0	98·0		$1{\cdot}4670^{18}$		0·54 (6 + 4)
(*R*)-Malaoxon	51·0	98·4	$+50{\cdot}0^{18}$ (c = 3, $CHCl_3$)	$1{\cdot}4635^{18}$	pure in the analysis conditions	0·54 (6 + 4)
(*S*)-Malaoxon	58·9	97·0	$-46{\cdot}6^{18}$ (c = 3, $CHCl_3$)	$1{\cdot}4692^{18}$	89·84	0·54 (6 + 4)
(*1RS,3RS*)-Isomalathion	47·0	98·4		$1{\cdot}5045^{18}$	—	0·35 (6 + 4)
(*1RS,3R*)-Isomalathion	34·9	97·2	$+42{\cdot}0^{18}$ (c = 2·5, $CHCl_3$)	$1{\cdot}5051^{18}$	—	0·35 (6 + 4)
(*1RS,3S*)-Isomalathion	28·8	95·7	$-38{\cdot}0^{18}$ (c = 2·5, $CHCl_3$)	$1{\cdot}5051^{18}$	—	0·35 (6 + 4)

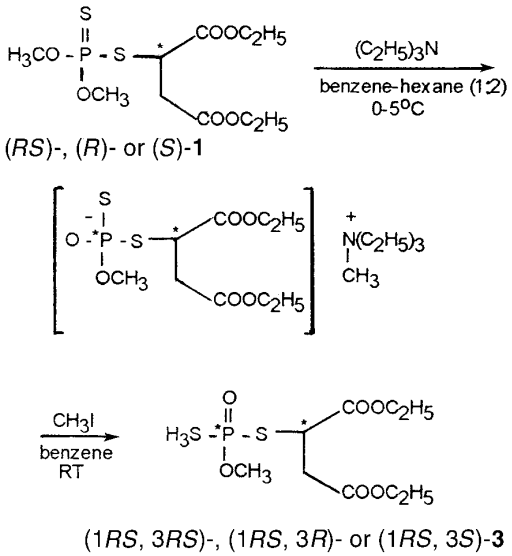


Fig. 7. Synthesis of isomalathion diastereoisomers.

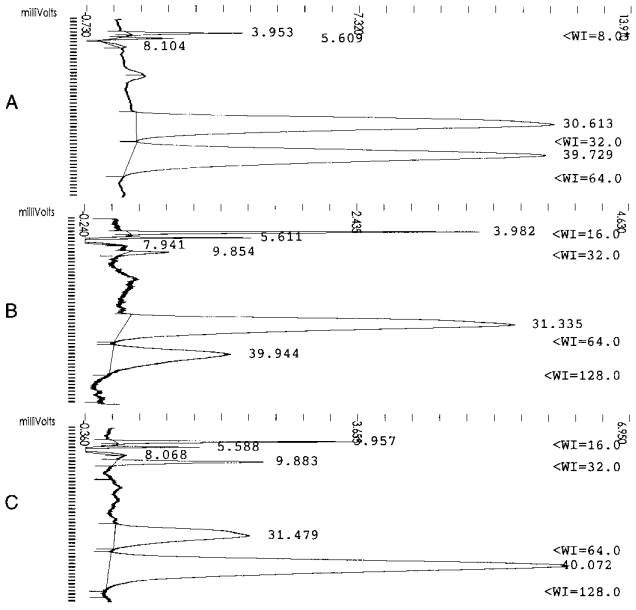


Fig. 8. Chiral HPLC resolution of (A) racemic isomalathion into diastereomers, and chromatograms of (B, C) isomalathion diastereomers synthesized from malathion enantiomers.

The physicochemical properties of the malaoxons obtained are given in Table 2.

The oxidation was carried out in hexane at room temperature. Intensive mixing of malathion-hexane suspension (1 + 10 by weight) allowed the inorganic acid to be added by dropwise safely. Reaction was continued for 5 h, with continuous removal of secreted nitrogen oxides under reduced pressure.

The mixture was then allowed to stand overnight. After work-up and purification of product by column chromatography on silica gel (eluant: hexane + ethyl acetate, 9 + 1, 8 + 2, 7 + 3 by volume) malaoxons were obtained in 51–58% yield. Figure 6 shows chromatographic resolution of malaoxon racemate into enantiomers, as well as (*R*)- and (*S*)-malaoxons.

2.3.3 *Isomalathion diastereoisomers*

We decided to synthesize pairs of isomalathion diastereoisomers with a view to studying the influence of chiral carbon configuration on toxicity towards mammals and insects. In order to obtain (*1RS*,3*R*)- and (*1RS*,3*S*)-isomalathion diastereomeric pairs, we used

TABLE 3

Acute Toxicity to Rats, and Arthropods of Malathion and Malaoxon Enantiomers, and Isomalathion Diastereoisomers

		LD_{50} (μg *per insect*)		LC_{50} (*mg litre*$^{-1}$)	
Compound	LD_{50} (*i.p.*) *for rats* (*mg kg*$^{-1}$)	*House-fly* (Musca domestica *L.*)	*Cockroach*[a] (Blatta orientalis *L.*)	*Granary weevil*[b] (Sitophilus granarius *L.*)	*Two-spotted spider mite*[c] (Tetranychus urticae *Koch*)
(*RS*)-malathion	2479	0·79	0·20	26	99
(*R*)-malathion	2286	0·53	0·15	32	49
(*S*)-malathion	2933	1·47	1·70	83	103
(*RS*)-malaoxon	28·6	0·95	0·057	18	10
(*R*)-malaoxon	23·12	0·47	0·045	12	13
(*S*)-malaoxon	47·96	2·02	0·224	24	10
(*1RS*,*3RS*)-isomalathion	47·74	2·45	0·17	936	1056
(*1RS*,*3R*)-isomalathion	36·98	0·75	0·08	812	491
(*1RS*,*3S*)-isomalathion	66·04	2·13	0·43	1000	613

[a] Topical application.
[b] Impregnated filter paper.
[c] Leaf dipping.

(*R*)- and (*S*)- malathions, respectively. Having the optically active malathion enantiomers, we transformed them into triethylmethylammonium salts of desmethyl malathions. This operation (giving 60% yield) generated chirality on the phosphorus atom.

After realkylation with the aid of methyl iodide, crude isomalathions were purified by column chromatography (eluant: hexane + ethyl acetate, 9 + 1, 8 + 2, 7 + 3 by volume). The course of isomalathion synthesis is presented in Fig. 7.

The rearrangement of malathion into isomalathion occured without the participation of the chiral, α-succinic carbon atom. However, bearing in mind the reactivity of its attached proton under the conditions used to obtain the ammonium salts of desmethyl (*R*)- or (*S*)-malathion, racemization is possible. Partial chiral HPLC resolution of synthesized pairs of isomalathion diastereoisomers showed that, although we started from 88% e.e. (*R*)-malathion and 90% e.e. (*S*)-malathion, each of the obtained diastereomers contained the second as impurity. In spite of this, toxicological research showed distinct differences in their modes of action.

The physicochemical properties of isomalathion diastereoisomers are presented in Table 2. Figure 8 shows the partial resolution of racemic isomalathion, and its diastereoisomers.

[^{1}H], [^{13}C], and [^{31}P] NMR spectra of the compounds obtained were found to be identical with those described in the literature.[10,11]

2.4 Toxicity

LD_{50} i.p. (for rats), LD_{50} or LC_{50} (for some arthropods species) values were determined for the synthesized malathions, malaoxons, and isomalathions using standard toxicological methods. In the case of all bioassayed compounds, (*R*) configuration on the chiral carbon atom caused the highest toxicity and (*S*) configuration the lowest.

Acute toxicity (i.p.) was estimated for 2·5-month-old Wistar female rats. LD_{50} was calculated by Thompson method using Weil tables.

The logarithmic-probit method of Finney[23] was used to estimate LD_{50} or LC_{50} for the arthropods tested.

The toxicological characteristics of the synthesized substances, expressed as LD_{50} or LC_{50} values, are given in Table 3.

3 DISCUSSION

Malathion enantiomers were obtained with good yield and stereoselectivity by the nucleophilic substitution reaction of diethyl (*R*)- or (*S*)-2-bromosuccinate with the *O*,*O*-dimethyldithiophosphoryl anion. Optimum reaction conditions were established (Section 2.3.1), and, with 1,4-dioxane as solvent and the ammonium salt of *O*,*O*-dimethyldithiophosphoric acid, resulted in quick and efficient progress of the process.

Malaoxon enantiomers were synthesized by way of thiono–thiolo rearrangement of (*R*)- or (*S*)- malathions, using 65% nitric acid as oxidizing agent.

Isomalathion diastereomeric pairs: (*1RS*,3*S*) and (*1RS*,3*S*) were obtained by desmethylation of malathion enantiomers with triethylamine, and remethylation of the desmethyl malathion salts with methyl iodide.

Physicochemical and toxicological properties of synthesized compounds were determined. It was concluded that the configuration at the succinic carbon atom controls the toxicity of all the stereoisomers obtained. Malathion enantiomers, as well as the racemate, are non-toxic to rats ($LD_{50} > 2000$ mg kg^{-1}), but it was noticed that (*R*)-configuration generates greater toxicity.

Both malaoxon enantiomers and isomalathion diastereoisomers are highly toxic to rats. The lowest toxic malaoxon (*S*) enantiomer was found to be 1,5-fold more toxic than the least toxic (*1RS*,3*S*)-isomalathion. The most toxic of the substances studied was (*R*)-malaoxon—its toxicity for rats was 100-fold higher than that of (*R*)-malathion and 1·6-fold higher than that of (*1RS*,3*R*)-isomalathion.

The arthropods tested were also more sensitive to the compounds with *R* configuration at the α-succinic carbon atom. (*R*)-malathion was 3-fold more active against house-fly than (*S*)-malathion, and (*R*)-malaoxon 5-fold more than its (*S*) antipode.

As in the case of rats, (*R*)-malaoxon LD_{50} or LC_{50} values were the lowest of all the tested compounds, indicating its higher toxicity.

Diastereomeric pairs of isomalathion have not shown such clear differences in activity against insects. This was probably due to the use in the tests of impure compounds—each of the diastereomers was contaminated by the other, as was shown by chiral HPLC analysis.

7 ACKNOWLEDGEMENTS

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